Supporting Information

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SI Materials and Methods

Cell Culture. HCT-116 O and HCT-116 N cells were maintained as described in *Materials and Methods* in the main text. NCI-H23 cells engineered to express inducible shRNA to MLH1 were maintained in RPMI media in the absence (MMR-proficient) or presence (MMR-deficient) of 500 ng/mL doxycycline (Sigma) as described previously (20). Ovarian cancer cell lines were obtained from American Type Culture Collection (ATCC) and cultured accorded using the recommended conditions.

Cell Assays. HCT-116 O H2B-GFP and HCT-116 N H2B-GFP cells were plated to Perkin-Elmer 96-well View Plates at 2,500 cells per well and incubated overnight. Cells were then treated with 0.5 μM Rh-PPO or left untreated. Triplicate samples were imaged every 3 h over 144 h, using an IncuCyte (Essen Bioscience). Nuclear count was determined over time for each field of view that was imaged. For cell viability assays, cells were plated to View Plates at 1,000–5,000 cells per well and incubated overnight. Cells were then treated with Rh-PPO in a dose–response for 3 d. Cells were fixed in 4% formaldehyde, permeabilized with 0.5% Triton X-100 in PBS, and then stained with DAPI (Sigma). The number of nuclei per well was counted using a Cellomics ArrayScan (Thermo Fisher) and used to calculate the percent viable cells relative to the number of cells from untreated samples.

An ELISA was used to evaluate the effects of compounds on transcription. HCT-116 N and HCT-116 O cells were seeded in 96-well plates at 4,000 cells per well and allowed 24 h to adhere. After 24 h, cells were treated with Rh-PPO (0–1 μM final concentration) or cisplatin (0–10 μM final concentration) at 37 °C under humidified atmosphere for 24 h. After the incubation period, an equal volume of 2 mM EU in prewarmed media was added to each well, to a final concentration of 1 mM EU per well. EU pulse labeling was carried out at 37 °C under humidified atmosphere for 1 h. After the labeling period, the media was removed, and cells were fixed with 3.7% formaldehyde in PBS (pH 7.2) at 50 μL per well for 30 min at room temperature. The fixident was removed, and wells were washed 1× with 200 μL PBS (pH 7.2). Cells were then permeabilized with 0.5% Triton X-100 in PBS (pH 7.2) (50 μL per well for 15 min) and washed with PBS. A working solution of the Click-It reaction mixture (Thermo Fisher) was prepared immediately before treatment while protected from light, according to the manufacturer's instructions. The click reaction was carried out at ambient temperature for 30 min in the absence of light. The wells were aspirated and washed with rinse buffer. The plates were protected from light, and fluorescence readout of the wells was carried out on a Flexstation 3 Multi-Mode microplate reader (495-nm excitation, 519-nm emission, 515-nm automatic cutoff; top-read).

Indirect immunofluorescence was carried out as described in *Materials and Methods* in the main text. Cells were plated to 96-well View Plates (Perkin-Elmer) and allowed to adhere overnight before treatment with compounds in a dose–response. After 24 h, cells were fixed in 4% formaldehyde, permeabilized with 0.5% Triton X-100 in PBS, and then blocked with 1× blocking buffer (Thermo Fisher). Cells were incubated with primary antibody to phosphorylated Chk1 serine 345 (Cell Signaling) at 1:100 in blocking buffer overnight at 4 °C, and then washed in PBS, incubated with a secondary antibody, anti-rabbit Alexa Fluor 488 (Thermo Fisher) and DAPI at 1:1,000 in blocking buffer for 30 min, and washed again in PBS. Stained cells were stored covered with PBS before imaging with a Cellomics ArrayScan (Thermo Fisher) to measure staining intensity and nuclear count.

At least three independent experiments, with duplicate samples, were performed. Data were analyzed with Graph Pad (Prism). A Student's *t* test was used to assess statistical significance.

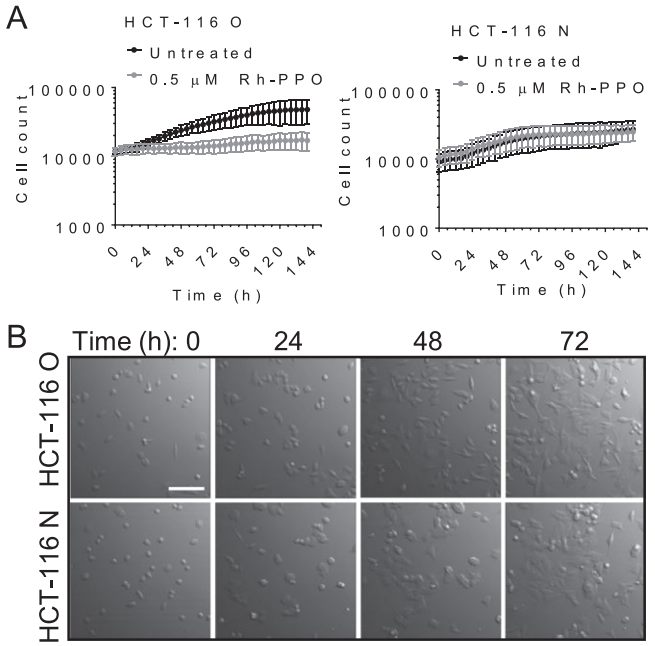

Fig. S1. Growth of HCT-116 O and HCT-116 N cells treated with Rh-PPO. (*A*) HCT-116 O H2B-GFP and HCT-116 N H2B-GFP cells that were untreated, or treated with Rh-PPO in a dose–response, were imaged every 3 h over 6 d. The graphs show the number of cell nuclei in a single field of view over time for triplicate samples. (*B*) Representative images of HCT-116 O and HCT-116 N cells over time are shown. (Scale bar, 100 μM.)

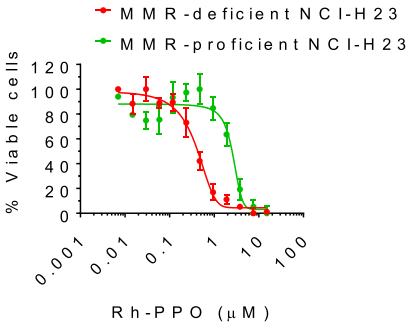

Fig. S2. Rh-PPO preferentially inhibits growth of NCI-H23 cells that are MMR-deficient. NCI-H23 cell lines containing a doxycycline-inducible shRNA to MLH1 were grown continuously in the absence or presence of 0.5 μM doxycycline, resulting in MMR-deficient and MMR-proficient isogenic cell lines, respectively. Cells were incubated with Rh-PPO in a dose–response for 72 h and then cell viability was assessed by a nuclear count assay. The graph shows cell viability for duplicate samples at each concentration.

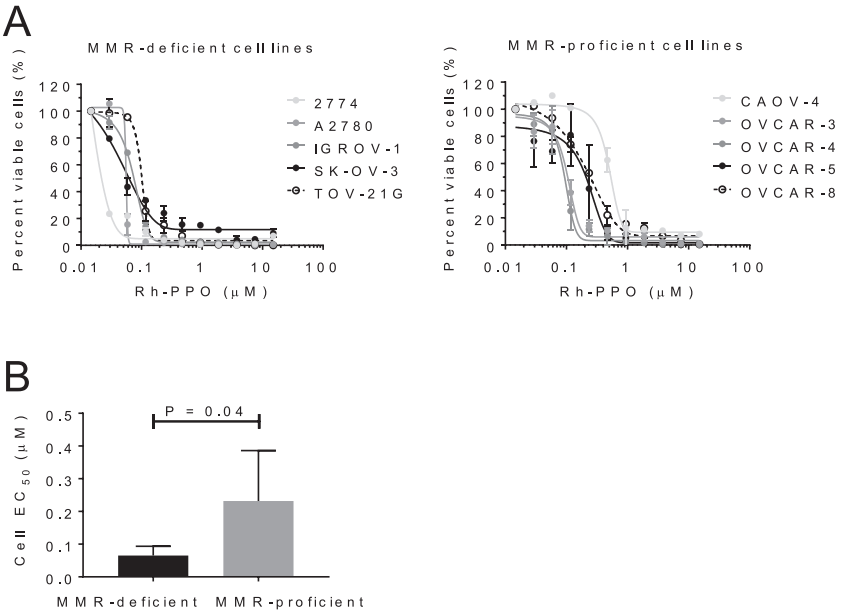

Fig. S3. Rh-PPO preferentially inhibits growth of ovarian cancer cell lines that are MMR-deficient. Five MMR-deficient cell lines and five MMR-proficient cell lines were treated with Rh-PPO in a dose–response for 72 h and then cell viability was assessed by a nuclear count assay. (*A*) The graphs show dose–response curves for cell viability of duplicate samples of ovarian cancer cell lines treated with Rh-PPO. (*B*) Comparison of the mean EC_{50} values for cell viability of MMR-deficient and MMR-proficient ovarian cancer cell lines (from *A*).

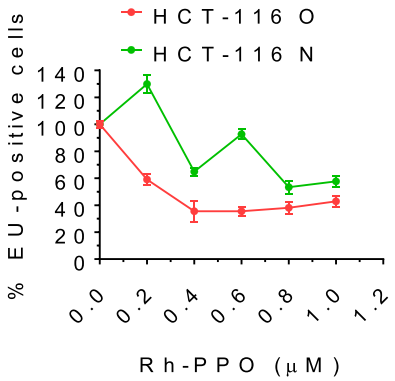

Fig. S4. Rh-PPO inhibits transcription. HCT-116 O and HCT-116 N cells were treated with Rh-PPO as indicated for 24 h, and cells were labeled with EU for 1 h before collection. EU incorporation was measured using a Click-It assay with fluorescent readout. Representative data from duplicate samples are shown.

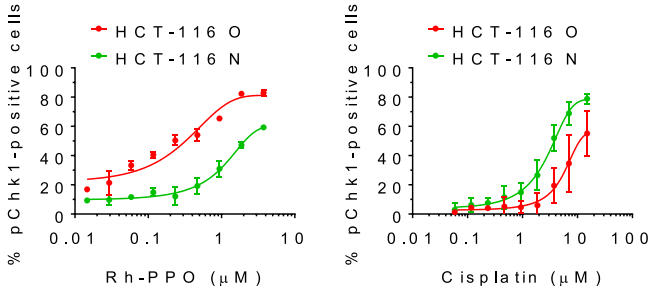

Fig. S5. Rh-PPO treatment activates the Chk1 checkpoint protein kinase. HCT-116 O and HCT-116 N cells were treated with Rh-PPO or cisplatin in a dose–response for 24 h and then cells were stained for phosphorylated serine 345 of Chk1 and counterstained for DAPI. The graphs show the percentage of cells from duplicate samples that stained positive for phosphorylated Chk1.